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Polymorphism of the Red Cell Acid-Phosphatase in Tuscany by Starch-gel Thin Layer Electrophoresis and Fluorogenic Substrate

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Summary. The red cell acid-phosphatase types were determined in 495 individuals from Tuscany, Italy.
The observed frequencies of the three alleles are: $ACP_1^A = 0.271$; $ACP_1^B = 0.665$ and $ACP_1^C = 0.064$.
The results obtained by starch-gel thin layer elcetrophoresis and 4 methyl-umbelliferyl-phosphate as fluorogenic substrate are very satisfactory for forensic purposes also.

Key words: Genetic enzyme polymorphism, ACP – red cell acid-phosphatase, gene frequency.

Zusammenfassung. Die sauren Erythrocytenphosphatase-Arten wurden bei 495 Personen aus der Toskana bestimmt.
Die beobachteten Frequenzen der drei Allelen sind: $ACP_1^A = 0{,}271$, $ACP_1^B = 0{,}665$ und $ACP_1^C = 0{,}064$.
Die erzielten Ergebnisse durch Stärkegel-Dünnschicht-Elektrophoresis und 4 methyl-umbelliferyl-phosphate als fluorogenisches Substrat sind auch für forensische Zwecke sehr zufriedenstellend.

Schlüsselwörter: Genetisch bedingte Enzym-Polymorphismen – saure Erythrozytenphosphatase, Genfrequenzen.

A polymorphism of the human red cell acid-phosphatase was detected in 1963 by Hopkinson et al. using a starch-gel electrophoresis and phenolphatalein diphosphate as substrate. Subsequently several studies of the distribution of this polymorphism in different populations have been undertaken. Furthermore, it has been utilized in many cases of disputed paternity.

According to Swallow et al. (1973), the method using methyl-umbelliferyl-phosphate as substrate, is the most sensitive. In 1975, this substrate was employed by Martin et al. with good results after electrophoresis on cellogel membranes.

A method based on starch-gel thin layer electrophoresis and methyl-umbelliferyl-phosphate as substrate for typing acid-phosphatase in haemolysates and in blood-

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Table 1. Red cell acid-phosphates phenotypes and gene frequencies in the population of Tuscany

	Phenotypes observed		expected		Gene frequencies
	n	%	n	%	
A	37	7,47	36,3533	7,3441	
BA	175	35,36	178,4128	36,0430	
B	221	44,65	218,9014	44,2225	$ACP_1^A = 0{,}271$
CA	19	3,84	17,1706	3,4688	$ACP_1^B = 0{,}665$
CB	41	8,28	42,1344	8,5120	$ACP_1^C = 0{,}064$
C	2	0,40	2,0275	0,4096	
Total	495	100,–	495	100,–	

$\chi^2 = 0{,}322658$ for 3 d. f. $0{,}95 > P > 0{,}98$

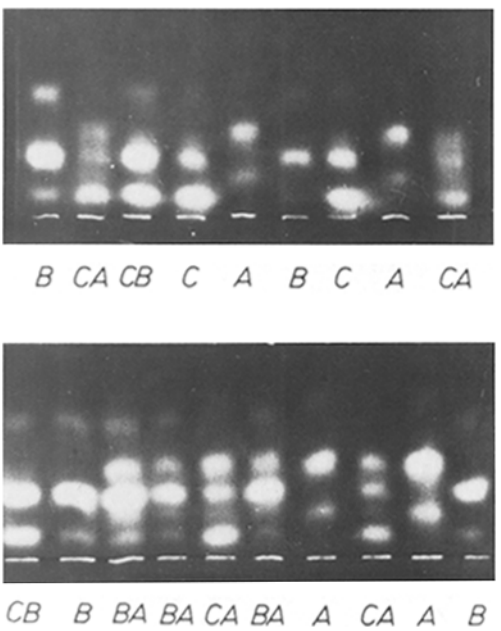

Fig. 1

stains has been recently reported by Wraxall and Emes (1976). In this study we have used this method, making some changes, for typing a population sample from Tuscany.

Materials and Methods

The sample included 495 random unrelated individuals. The haemolysates were prepared only by freezing at –20°C and then by thawing the saline washed red-cells. They were tested within 3–4 days.

A 12 % starch-gel (Biotest – Frankfurt am Main), 1 mm thick, was prepared according to Wraxall and Culliford (1968).

The buffers were: bridge buffer – 0,245 M sodium dihydrogen phosphate and 0,150 M trisodium citrate, pH 5,9; gel buffer: 1 in 100 dilution of bridge buffer.

The haemolysates were introduced into the gel, at about 6 cm from the cathodic bridge, soaked into cotton threads, approximately 8 mm long. The electrophoresis was carried out at

300 Volts (mA 15–17 → 20–27) for 4 hours on an alluminium cooling plate, through which water at a temperature of between 5°C and 10°C was circulating.

The reaction mixture was: 4 mg of 4-methyl-umbelliferyl-phosphate were dissolved in 10 ml of the reaction buffer (0,05 M citric acid and 0,05 M sodium hydroxide at pH 5). Pieces of Whatman 3 MM filter paper cut to the size of the gel were soaked in freshly prepared substrate solution and then laid on the uncut surface of the gel. The plate was incubated at 37°C for about 60 minutes.

The sites of acid phosphates activity could be seen fluorescing brightly under a long-wave ultra-violet lamp (366 nm).

Results and Discussion

The electrophoretic separation of the six common phenotypes (with some repetitions) are shown in Figure 1.

The interpretation of the electrophoresis patterns has presented hardly ever any difficulties. In most samples there is present a fast-moving anodic isoenzyme. Sometimes, in older haemolysates, there can appear a weak band in anodic position just beyond the „b" isoenzyme.

The method requires an electrophoresis of only 4 hours at 300 Volts and a short incubation time.

Owing to the use of methyl-umbelliferyl-phosphate as substrate, this method is very sensitive and the isoenzymes remain visible for a relatively long time. Therefore, it has some advantages in forensic haematology.

The phenotypes in our population sample and the calculated gene-frequencies are given in Table 1.

The results agree with the expectation of a 3-allelic model; the values according to the Hardy-Weinberg equlibrium.

Comparison with some other Italian population genetic studies (Marigo and Cortivo, Modiano) shows no significant differences.

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